June 7, 1950.

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Dear Tom:

Thanks very much for your thoughtfulness in sending a copy of your thesis. I don't know whether Stern will send it to me for reference, but if he does, I will certainly endorse it very highly. There are a few small points that you might like to hear about, and I am going to recite them on the assumption that your sending the MS justifies it. I haven't read the MS as critically as I hope to, in order to send you this reply before you might leave Columbia.

The first point that struck me was the title. I got the same reaction from other people here. Wouldn't "Kinetics of " or "Kinetic studies of..." be more meaningful than "Quantitative"? As an aside, your paper is the kind of work that should occupy space in Biometrics in addition to the more biostatistical papers there. Would you consider sending it there? I am not going to bother prefixing "Don't you think..." to the following:

P. 1 What about Tatum! (Tatum,E.L. and Lederberg,J., 1947 Gene recombination in the bacterium Escherichia coli. 53: 673-684. J. Bact.)

p.2 I don't think that Ryan and Schneider 1949 went so far as to suggest that syntrophy plus reversion accounted for any prototrophs. They indicated that microcolony formation should be taken into account in considering those "pregnant" experiments, whatever that means! Even with considerable syntrophy could you get enough of a population of parents for measurable coincidental reversion? Considering your negative evidence, and the weakness of the hypothesis, you may have given it too much emphasis.

p.3 E. coli. In view of complete absence of evidence for sexual differentiation, why bring it in gratuitously? The arguments still apply with respect to the detectable prototrophs.

p. 4 Can you regard the agreement of kinetic data (in a pair of strains selected for study on this basis) as crucial evidence for syngamy?

Line 2. So have a good many other people (Delbruck, e.g.,) and myself. This can be phrased a bit more fortunately- Kamm ( ) has also initiated an examination of this relationship...

p.5 and ff. A detailed theoretical development of this sort is desirable for a thesis, but should be condensed for publication. Only one derivation need be given; in fact, a standard reference (Hinshelwood?) to second order reactions might suffice. A general expression of the form $Z = K n_1 n_2$ is all that is essential, where Z is the rate of prototroph formation, and n the concentrations

9-10. (My reaction to Haas et al. is that the evaluation of Z is only meaningful if kinetic studies have been done which show the constancy of K with variable n's, conditions, etc.)

12. May be confusing. Do you mean to imply the existence of triparentage? The second paragraph should perhaps be expanded. Unless you show that other hypotheses cannot give you $Z = k n_1 n_2$ without improbable auxiliary hypotheses, you don't have very crucial evidence for syngamy.

14. What special precautions? Do you mean particular care?

15. "Student's or The Student's t test "

16. Relationship for relation.

18. Kinetic equation for kinetics equation. Headings, like this one, should be in roman capital for main sections; started with italic capital for subsections — at least this has been practice in Genetics.

23. You start out, properly, by suggesting increased yields with supplemented agar as a secondary method for determining linkages. It is certainly not a satisfactory absolute method. My Table 4 (1947) agrees with your plate data in suggesting that L- are about 10% of prototrophs and T- about 25%. The increased yield is not a satisfactory method; the colonies should be picked and tested. Even then, it is difficult to evaluate syntrophy and single reversion. The former has always been prominent in my experiments with L- and T-supplemented minimal. Your statement about the absolute cross over values is correct, but has little to do with the foregoing, which concerns relative values (p. 515 "1947"). p/24: "Mapping ... random or total recovery... Why not make a separate paragraph.

24. Lines 1-12? You don't mean it.(12-15: incontrovertible (but unlikely — I should find unusually fertile f-1 combinations, and don't.)). 679-680 and Y-24 are both descended from K-12. The a priori probability that they are different mating type is what you think the mutation rate may be. The five isolates do not constitute independent tests. Why not forget it.

30. Can't a collision factor, at least approximate, be determined for the experiment of Fig. 11? I will try to estimate it assuming a kinetic energy equal to thermal plus translation of shaker. This will be an overestimate, but may be worthwhile.

Table 1. 679-680 was produced by and received from Tatum (1945 X-ray induced mutant strains of Escherichia coli. PNAS 31: 215-219.) (Sidenote: Bernie Davis doesn't believe that 58-161 has a biotin requirement- like your Y-24. I think the amino acids spare the biotin requirement to the point where it is very difficult to detect.

Table 2. A good fit, but I don't think it is needed in the publication. I'm willing to take your word for the agreement with expectation, and can't use the data for anything else.

Table 3. Column headings should be f (?), rather than the equation used in the calculation, which should be in footnote. In the second part (which does not really add a great deal), the t- and 1- , etc., are inferred by subtraction, which should be stated. I presume the t tests were done on the data of the upper part.
The figures and the captions are very good. Most of the titles are a bit confusing, however: e.g., "linear law". Fig. 3 What cross?, what numbers?

Fig. 4 Which cross. Or does the proviso 679-680 x Y-26 apply to the figures?

Fig. 8 "t" is an unfortunate symbol, because of possible confusion with the statistical test. This may sound foolish, but it took me several minutes to grasp the figure. The overlapping coordinates are also confusing.

Fig. 13 Not a fair test of syntrophy which is concentration-dependent. How about using somewhat higher concentrations of cells, and cutting out the prototroph colonies. I can't compare my impressions in detail with yours, however, as I made a very limited use of well-washed agar.

p. 28 agar layer experiment: "me too", but I don't think this is mentioned in print anywhere. Davis' sterile filter experiment is very elegant, and I think he plans to publish it. It may be appropriate to refer to it (with his consent).

GENERAL (Bull-session)

a) Figures. I think you may have too many for an effective presentation, but it is hard for me to suggest which ones can be eliminated. Some in the series 3-7 might be.

b) Conclusions. Not much to argue with, except that, as I mentioned above, some more consideration should be given to the work as a "critical experiment" disqualifying other mechanisms than syngamy. It is amazing how many forms such alternatives can take (especially transformation), and I don't think anything short of a cytological demonstration of fusion is going to be satisfactory. I think that the concept of transforming factors merges with that of syngamy to the point where no indirect evidence can be decisive. Your work is especially important as it may put any sense for the zygote, estimates of its frequency etc., on a sound basis, and may make it possible to look for it intelligently. With your information, it may be possible to concentrate potential zygotes by physical methods (differential centrifugation or other transport), as a basis for cytological study. I assume that you have had this in mind, and hope that you plan to do something about it at Caltech.

c) Speculations. 1) Age, hormones, etc. There may very well be something to it. I think that Cavalli and Westergaard are looking for same, but your kinetic work puts you way ahead.

2) What do you think of the possibility that the mixtures result in more or less non-specific aggregations of the two parental cell types, which do not actually form zygotes until later. That such auto-agglutination does occur to a limited extent in K-12 is apparent from dilute platings of Lac- and Lac+ mixed cultures on EMB lactose. I think that some such aggregations survive shaking but are distributed by shearing with a glass spreader. The evidence for this is that some replicate platings of a Lac -/+ mixture have shown a very high variance for the ratio of types from plate to plate, as well as for the numbers. But the main evidence is the occasional sectored colony on dilute plates. I think your other suggestions on p 26 are more likely, but this might account for the saturation effect. Such aggregation would of course make both syngamy and transformation compatible with your kinetic data, and illustrate one direction of the gradual merger of the two notions. The possibility is cogent enough, I think, to warrant study along the lines of your analysis, using platings of mixed cultures on EMB lactose agar.

d) references — as a charter member of MGB, I think it is a bad precedent to refer to anything in it, except as (e.g.) Ryan, F.J., private communication, preferably in the text rather than the bibliography.

After all this detailed criticism, I want to congratulate you for your degree, and for a very good job. Most of my comments concern the manner of presentation, rather than the content, and many of them are open to question. I hope they can be of some use to you, but it occurs to me now that you may have sent me an earlier draft, and not the final paper. But I enjoyed reading the MS too much for this to have been a waste of time.

Esther and I will be leaving here in about two weeks, and will turn up at Caltech probably during the last week in July, so I hope we will have a chance to see you there.

With wishes for the very best (not of luck, but earned accomplishment),

Sincerely,

Joshua Lederberg